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DEVELOPMENT OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT AND DURUM USING MICROSPORE/TISSUE CULTURE

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Development of Fusarium Head Blight Resistance in Wheat and Durum using Microspore/tissue culture.

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ABSTRACT

In this research we set out to test deoxynivalenol (DON) as an *in vitro* selection tool for Fusarium head blight (FHB) tolerance and to identify/incorporate novel sources of resistance into durum. Repeated experiments confirmed that DON was not suitable for *in vitro* selection. Novel resistance in four *Triticum dicoccoides* "TG" accessions was confirmed in both greenhouse and field trials. The ferulic acid (FA) content was highest in TG accessions when compared to durum varieties, suggesting that FA maybe the cause of elevated resistance. Durum lines with an introgressed resistance gene *(Fhb1)* were also evaluated and found to have improved tolerance. These lines will be useful parents for further backcrossing to improve agronomic performance.

1.0 INTRODUCTION

Fusarium Head Blight (FHB) is a fungal disease of small-grain cereals including wheat, durum and barley. FHB infection causes reduced yield, and reduces the physical quality of grain, with lower test weights and a correlated reduction in flour/semolina yield. Grading tolerances for FHB-damaged kernels are low, and substantial grading losses can occur in susceptible varieties. Genetic resistance is required to ensure that the yield and quality of spring and durum wheat produced in Saskatchewan is maintained. Given the increasing evidence of the negative effects of DON on human health, wheat breeders are recognizing the need to not only incorporate Type I (resistance to infection) and II resistance (resistance to fungal spread), but to also incorporate Type III (resistance to mycotoxins) to provide increased food safety. Unfortunately, breeding for FHB resistance is extremely challenging as resistance is genetically complex and expression is highly influenced by the environment (McCartney et al. 2004). In addition, our breeding programs do not have access to endemic nurseries in Saskatchewan for screening early generation materials. Thus an alternative screening technique that allows screening of a large amount of plant material is needed.

Several *Fusarium* species can cause FHB, with *Fusarium graminearum* being the most important of these species in western Canada. *F. graminearum* produces harmful mycotoxins (trichothecenes) which have negative health effects for animals (particularly swine) and humans. The most prominent trichothecene produced by *F. graminearum* is deoxynivalenol (DON). DON and other trichothecene are thought to play a significant role in the *Fusarium* diseases of cereals (Bai and Shaner, 2004; Desjardins, 2003). In fact disruption of DON biosynthesis in *F. graminearum* has been shown to result in reduced virulence on wheat by limiting the ability of the fungus to colonize beyond the inoculated spikelet (Proctor et al., 2002). These results suggest that DON maybe a critical factor for the spread of FHB within a spike (Bai et al., 2001; Proctor et al., 2002). Based on these results we hypothesized that selecting plantlets with DON resistance would result in indirectly selecting for increase resistance to FHB infection and spread.

In this study, we propose using microspore culture in combination with selection for DON resistance *in vitro* as this strategy has been reported to be an effective selection tool (Bruins et al. 1993). Microspore culture was chosen as it is a rapid method to obtain

homozygous lines in wheat breeding programs (Liu et al. 2002). Much effort has been spent to improve anther culture techniques in wheat and recently systems for isolated microspore culture with large potential for plant production have been reported (Liu et al. 2002). Given the variable nature of tissue culture experiments, the first objective of this research was to develop a suitable microspore culture system for wheat, and more specifically to test the possibility that DON could be included in the microspore culture protocol as a means to select microspore-derived plantlets that had the ability to tolerate high concentrations of deoxynivalenol (DON) *in vitro*. The idea is that by selecting for DON resistance, we could indirectly select for elevated levels of field resistance to FHB. This selection protocol could then be used by breeders as a tool to increase the frequency of FHB resistance genes in wheat prior to expensive field testing/FHB screening. The major advantage of a workable *in vitro* selection protocol is that a larger number of genotypes can be screened under controlled conditions prior to expensive field testing.

The development of durum wheat varieties with good FHB tolerance has largely been hindered by a lack of effective resistance genes. One strategy available to durum wheat breeders is to exploit existing variation for FHB resistance in the primary gene pool of durum wheat. A number of studies have shown that variation for FHB resistance in wild relatives of durum wheat does exist (Buerstmayer et al. 2003), which could be used as a useful source of FHB resistance for durum wheat breeding programs. Thus, an additional objective of this research was to identify and characterize alternative sources of FHB resistance for durum wheat which could be used by western Canadian durum wheat breeders as an effective source of FHB resistance. A second strategy to improve FHB resistance in durum wheat is to transfer effective resistance genes from bread wheat into a durum wheat genetic background. In this study, we successfully transferred *Fhb1*, which is located on chromosome 3B (Cuthbert et al. 2006) from ND2710 into a durum wheat genetic background and report the performance of these lines

2.0 MATERIALS AND METHODS

2.1 Optimization of a Suitable Microspore Culture Protocol:

Prior to conducting selection experiments, the wheat microspore culture system was first optimized for a sampling of genotypes used in the selection experiments. Given the variable

nature of wheat genotypes in responding to microspore culture (Liu et al. 2002), we felt it was necessary to test and adapt the protocol for Canadian wheat genotypes.

Tiller Collection and Spike Pre-treatment:

Wheat microspores were isolated from wheat plants at different stages of phenological development to determine appropriate staging for extraction of embryonic microspores (embryonic microspores are those that have the potential to be generated into plants). The donor shoots were collected when the anthers of the middle-part of the spikes contained the microspores at either the mid uni-nucleate stage or the bi-nucleate stage. At appropriate sampling times, wheat varieties included AC Barrie, CDC Teal, CDC Bounty, and durum wheat AC Avonlea. Fresh tillers were cut two nodes below the spike, and placed in a clean container with distilled water. All leaves were removed by cutting at their bases. Collected tillers were placed in an autoclaved sterile flask, containing 50 mL of pre-treatment media (Liu et al. 2002). The open end of a plastic bag was placed over the spikes and sealed around the flask to limit microbial contamination. To determine appropriate stress treatments to induce microspores into embryonic development, flasks were placed in an incubator at varying temperatures (5°C, 15°C and 33°C) for 48 hours.

Microspore Isolation and Culture:

Under sterile conditions, induced wheat spikes were surface sterilized with 70% (v/v) ethanol and covered in Kimwipe saturated with 70% ethanol and allowed to dry for 30 minutes. Using scissors and forceps, the spikes were isolated from the sheath, placed into sterile Peti-dishes and florets cut from the spike rachis. Excised florets were placed into a blender cup with 40 mL of 0.3 M mannitol. The mixture was blended at approx. 2200 rpm for 20 seconds. The blended slurry filter sterilized (100 uM screen) into a 250 mL flask. The blender cup was rinsed twice with 5 mL of 0.3 M mannitol and filtered through the same filter. Filtrate was transferred to a sterile centrifuge tube and spun at 100 *g* for 3 minutes. The resulting supernatant was decanted and the pellets from each tube resuspended with 2 mL of 0.3 M mannitol. Microspores were separated using gradient centrification by applying the resuspended pellets onto the surface of 5 mL of 0.6 M maltose solution in a 15 mL falcon tube and centrifuged at 100 *g* for 3 minutes. Microspores were collected from the middle band and microspore concentration determined using a haemocytometer. Microspores were concentrated at a density of approx. 10^4 microspores per mL in NPB99 culture medium (Liu et al., 2002). Finally, each 5 mL of the final microspore

containing solution was transferred onto a 60 x 15 mm petri-dish. Ovule co-culture has proven to be effective at increasing the frequency of embryogenic microspores in wheat (Liu et al., 2002). Ovules for co-culture were extracted from CDC Bounty using sterile technique and plated at a density of 5 ovules per mL of NPB99. The Petri dishes were sealed and incubated at 27°C in the dark for development.

Embryoid regeneration

Once embryoids (microspore-derived callus) had developed from microspores, they were transferred to 60 x 15 mm Petri-dishes containing sterile, solid MS regeneration media containing 0.3 M sucrose at a density of 20 to 25 embryoids per dish. Embryoids were incubated under continuous fluorescent light at 24°C. In approximately 3-4 weeks, green plants were transferred to soil and grown to maturity in the greenhouse. Fertility of plants was recorded as a means of assessing spontaneous chromosome doubling.

2.2 Tissue Culture Selection Experiments:

Experiments were conducted to optimize the concentration of DON that would be required for effective differentiation of microspores derived from resistant and susceptible spring wheat and durum cultivars. Experiments were conducted using a range of varieties including: ND2710 (spring wheat with FHB resistance), AC Barrie (intermediate FHB resistance), Snowbird (hard white wheat susceptible to FHB), CDC Teal (spring wheat susceptible to FHB), and Strongfield (durum wheat susceptible to FHB) (McCartney et al., 2004).

For selection experiments, deoxynivalenol (DON; Sigma Catalogue No. D0156), was included in the NPB99 media (post-induction) at concentrations ranging from 0-1000 mg DON L^{-1} along with induced microspores. In the original proposal culture filtrate of *F. graminearum* was to be used as the selection medium, but since DON itself has been reported as the virulence factor, we felt that this would be most suitable. In addition it is stable at room temperature (we are not certain about the stability of compounds in culture filtrate) and much easier to quantify (i.e. culture filtrate contains a host of compounds and it would be difficult to assess concentrations of all compounds for use in repeated experiments). Twenty one days after treatment, microspores in NPB99 media were placed in gridded petri-dishes (to facilitate counting) and examined under an inverted microscope to determine the percentage of embryonic microspores developing into callus) based on counting of 1000 embryonic microspores. Two

experiments were conducted as a completely random design with two replications in each experiment. Data presented represents the mean (±SD) of each DON treatment.

A second set of selection experiments were conducted by including DON in the regeneration medium once plantlets had developed from embryoids. In those experiments, germinated embyroids were allowed to grow for 3-4 weeks until root and shoot development was clearly evident. Developing plantlets with healthy roots and shoots were then transferred to fresh MS media containing variable concentrations of DON, ranging from 0-1000 mg L^{-1} and were grown at 23°C under continuous light for 25 days. Each MS plate contained 25 developing plantlets. One month after plating, the number of surviving plantlets was determined. Three experiments were conducted as a completely random design with two replications in each experiment. Data was analyzed using a general linear model (GLM) analysis of variance where genotypes and concentrations of DON were considered fixed effects and experiment and replication effects considered random effects. Data was combined prior to analysis and is presented as the least square means of the three experiments. A least significant difference (LSD) was calculated at a 5% significance level for comparison of treatment means. Wheat varieties in the study included ND2710 (spring wheat with FHB resistance), AC Barrie (intermediate resistance), CDC Teal (spring wheat susceptible to FHB), Strongfield (durum wheat susceptible to FHB) and Maringa, a variety with putative resistance to DON (Type III resistance (Somers et al. 2003).

In addition to haploid plantlet selections, a third tissue culture selection experiment using coleoptile resistance was conducted in the presence of increasing DON. Varieties tested included spring wheats CDC Teal, AC Barrie, Snowbird, ND2710, and Maringa, and durum wheats AC Avonlea, Strongfield, Commander and DT735. Surface sterilized seeds were placed in petri-dishes and imbibed at 15°C for 10 days. When the seeds had germinated (visualization of the radical), 15 germinated seeds were transferred to petri-dishes containing solidified MS media. Based on the microspore plantlet derived experiments, plates were spiked with 0, 5, 10, 15 and 20 mg L^{-1} of DON. Plates were then placed in a growth incubator at 21°C in the dark. Coleoptile lengths were measured five days post DON treatment. Four plates per variety were examined in a completely random design.

2.3 Greenhouse testing of Novel Durum Wheat Accessions for FHB Resistance

Four accessions of *Triticum turgidum* var. *dicoccoides* with putative FHB resistance namely TG3487, TG13475, TG42074, and TG13205 were tested for resistance to FHB in two greenhouse trials in 2004. ND2710, AC Barrie, CDC Teal, and Strongfield were included as check cultivars. In addition, we crossed each of the four accessions with Strongfield and evaluated F_1 plants for FHB reaction using methods described above to confirm physiological resistance and to determine if resistance was heritable. For all greenhouse trials, a completely random design with four replications was used for each experiment, with the exception of the F_1 plant screening, were five F_1 plants were screened. To evaluate Type II resistance, procedures based on Jin et al. (1999) were used. Briefly, *F. graminearum* isolates were grown on cornmeal agar for 5-7 days. Cleaned canary seed was soaked in distilled water for 24 h, spread in a thin layer in a glass petri-dish and autoclaved. Autoclaved seed was inoculated with agar pieces of *F. graminearum* and cultured for 10-12 days at room temperature under continuous fluorescent light. Colonized seeds were air-dried and stored at 4°C until use. Prior to inoculation, *F. graminearum* infected canary seed was soaked in distilled water for 20 minutes. At anthesis (anthers just beginning to extrude), plants were inoculated by placing a single infected canary seed in a single primary floret in the centre of the spike. Four heads were inoculated per wheat cultivar. Following inoculation, plants were placed in moist chamber for 24 h and 21 days after inoculation, accessions were rated for infection using the scale presented in Table 1.

Table 1. Greenhouse Rating Scale for Assessment of Fusarium infection of Wheat plants.

Score	Description
0	no infection
1	only inoculated floret infected
2	only inoculated spikelet infected
3.0	inoculated spikelet and rachis infected
3.2	inoculated spikelet and one adjacent spikelet infected
3.4	inoculated spikelet and two adjacent spikelets infected
3.6	inoculated spikelet and three adjacent spikelets infected
3.8	inoculated spikelet and four adjacent spikelets infected
4	half spike infected
5	whole spike infected

All data was subjected to an analysis of variance using PROC MIXED of SAS with varieties considered fixed effects.

2.4 Field Testing of Durum Populations for FHB Resistance:

The four TG accession, and sets of breeding families developed by backcrossing the TG lines into DT735 were evaluated in field trials. DT735 is a breeding line from the AAFC-SPARC breeding program that has consistently displayed improved FHB tolerance compared to registered durum wheat cultivars. In more detail, each TG accession was backcrossed once to DT735 and the F_1 seed increased. The resulting F_2 populations were increased in January 2005 and 300 (range 288-312) single plants were selected from each population to produce $F_{2:3}$ families. In 2005, 100 randomly selected $F_{2:3}$ families from each of the four populations were evaluated for resistance to FHB at Carman, MB. Each family, together with their parental lines and a minimum of four repeated rows of each of the check cultivars, were grown in 2.5 m rows. Check cultivars included CDC Teal (susceptible to FHB), AC Morse (susceptible durum wheat), DT735 (moderately resistant durum), FHB37 (spring wheat with excellent resistance to FHB), and each of the four accessions. Rows were inoculated with *Fusarium graminearum* at anthesis and again four days after the first inoculation date. Rows were mist irrigated after each inoculation to promote disease development. Approximately 21 days after inoculation, each family was rated for incidence and severity of FHB infection and a FHB index (incidence x severity) was calculated as a measure of resistance. Observations were used to calculate a frequency histogram of FHB indices for each of the four $F_{2:3}$ populations. Data from check cultivars is presented as the mean of four observations.

Results from greenhouse and field house testing confirmed that TG3487 has better Type II resistance than the other three accessions. As such, a fifth population consisting of approx. 500 F2:5 lines with pedigree DT712//TG3487/2*DT735 was developed using single seed descent and was evaluated for FHB resistance in 2006 field trials. A population of approx. 400 F5 lines derived from the cross DT712/DT735 was also evaluated for comparison. Check cultivars, inoculation and disease scoring was performed as outlined above for the 2005 field trials.

2.5. Molecular Transfer of *Fhb1* from ND2710 into durum wheat

Although not part of the original proposal, a small project was continued with the objective of incorporating the *Fhb1* resistance gene from spring wheat variety ND2710 into durum wheat and to evaluate field resistance. In 2004, a set of F_5 lines derived from the cross DT513/ND2710//Strongfield were first screened for leaf rust resistance and surviving lines then screened using markers *gwm533* and *gwm493*, which flank the *Fhb1* locus. PCR and DNA electrophoresis protocols were exactly as described previously (Matus-Cadiz et al. 2006). Six F_6 lines that contained the *Fhb1* locus were increased and evaluated for FHB tolerance in the Carman FHB nursery as described above and in replicated yield trials at Saskatoon. Check cultivars included AC Avonlea, AC Morse, Strongfield, and AC Navigator, all durum wheat varieties.

2.5 Ferulic Acid Determinations

Previous studies on maize indicate that phenolics in grain may be involved in FHB resistance in wheat (McKeehen et al., 1999). The Four TG accessions along with checks DT735 and Strongfield were selected to perform an initial study to determine differences in ferulic acid content. Samples were collected from plants grown in the Phytotron at 24°C/16°C day/night temperatures and a 16/8 hour photoperiod regime. Three replications of each accession and check cultivar were grown in a completely random design. Wheat spikes were collected at 15 days post anthesis (dpa) and again at physiological maturity and the grains removed for analyses. Sampled grains were finely ground on a Udy Cyclone sample mill fitted with a 0.5 mm screen. Phenolic acids were extracted from 200 mg of ground samples and measured using gas chromatography as described previously (Abdel-Aal et al. 2001). Purified ferulic acid was used as internal standard for quantification purposes.

3.0 RESULTS

3.1 Development of a Microspore Culture System for Wheat

A number of studies have developed and tested suitable microspore culture protocols for developing doubled haploid wheat plants. The majority of studies however have focussed on winter-habit wheats which are generally regarded as being less recalcitrant than spring wheat

types. Furthermore, strong varietal differences among spring wheats exist for performance in tissue culture (Bruins et al. 1993; Lie et al. 2002). As such, existing protocols were first tested to determine there applicability to Canadian spring wheat cultivars, a prequiste for testing *in vitro* selection. We first set out to determine appropriate sampling time to maximize the number of embryogenic microspores. Liu et al (2002) reported that embryonic microspores could be morphologically differentiated based on size of the cell, the presence of larger vacuoles, and intercellular star-shaped structures. In our studies, it was quite easy to identify embryonic microspores as they were nearly 2X the size on non-embryonic microspores. Microspores collected at the mid-late uninucleate stage (Figure 1) produced a higher frequency of embryogenic microspores in all varieties examined (Table 2).

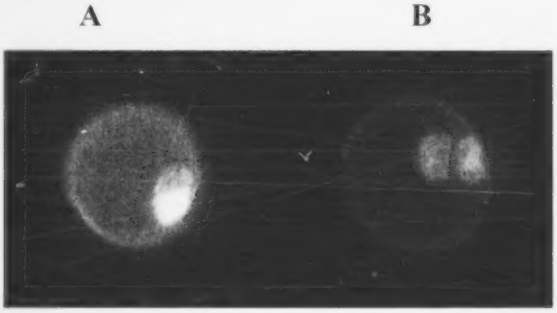

Figure 1. Uninucleate (A) and binucleate (B) microspores collected from CDC Teal at the booting stage (A) just prior to spike visualization and just prior to complete heading (B).

However, AC Avonlea had the lowest proportion of embryonic microspores compared to the spring wheat varieties, and this difference was statistically significant. It should be noted that microspore development could not easily be judged based on plant phenological development and sampling examination of microspores of anthers at different stages of phenological development was necessity to determine the appropriate stage for microspore sampling. Furthermore we made a general observation that this stage of microspore development was short lived (approx. 2 days), making sampling time critical. However, generally mid-late uninucleate microspores could be obtained from all cultivars examined just prior to spike emergence from the boot.

Table 2. Percentage of embryonic microspores at two stages of microspore development. Data represents the average (standard deviation) from three separate microspore extractions.

Cultivar	Mid- late uninucleate	Bi-nucleate
CDC Teal	60(6)	0(0)
AC Barrie	52 (7)	2(1)
CDC Bounty	67(10)	0(0)
AC Avonlea	26(5)	0(0)

The induction of microspore embryogenesis can be triggered by temperature shock, starvation, or osmotic treatments, resulting in disruption of the normal pollen maturation pathway in favor of somatic cell divisions (Liu et al. 2002). We evaluated the effects of different temperatures on embryogenesis and found that for the spring wheat varieties evaluated an induction temperature of 33°C was better at inducing embryogenesis in the majority of cultivars evaluated (Table 3). However, differences between varieties were clearly evident.

Table 3. Average % embryonic microspores (standard deviation) at three different spike pre-treatment induction temperatures.

Variety	Induction temperature	% Embryonic Microspores
CDC Teal	5	2 (2)
	15	26 (3)
	33	54 (6)
AC Barrie	5	3 (2)
	15	38 (4)
	33	64(7)
CDC Bounty	5	4 (1)
	15	34 (3)
	33	52 (6)
AC Avonlea	5	0 (0)
	15	2 (1)
	33	23 (3)

Of the spring wheats, AC Barrie had the highest frequency of embryonic microspores at 33°C. As in the previous experiment (Table 2), AC Avonlea had the lowest percentage of embryonic microspores, but 33°C was still the best temperature for microspore induction in that variety. Induction at 5°C resulted in few embryonic cultivars, regardless of cultivar evaluated (Table 3). A large portion of the microspores pretreated at 33°C were nearly double in size to those treated

at 5°C. Although it appears that % embryonic microspores are low (23-64%), thousands of microspores are obtained from any given extraction. Thus, even a small proportion of embryonic microspores will result in sufficient materials for generation of haploid plantlets. Using the 33°C induction temperature, we were able to regenerate green plantlets on standard MS media (Figure 3) for further study.

The plantlets derived from microspore culture were presumed to be haploid, since they were derived from microspores, which are haploid cells. However, spontaneous doubling of chromosomes is a common phenomenon in microspore culture experiments (Lie et al. 2002 and references there in). As such, a three-replicate experiment was conducted to determine if difference in the frequency of spontaneous doubling were evident among the spring and durum wheat varieties evaluated.

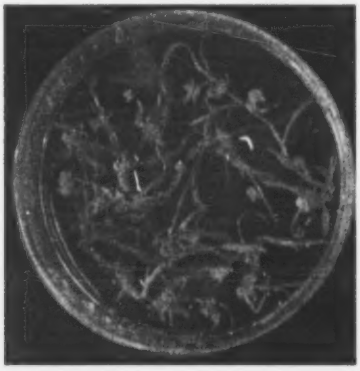

Figure 3. Green-plantlets derived from microspores obtained from CDC Teal tillers pre-treated at 33°C for 48 hours.

Spontaneous doubling of chromosomes was measured based on plant fertility (n=25 plants per replication from separate microspore extractions) as haploid plants would not produce viable gametes for fertilization to occur. Differences in fertility were evident among the spring wheat and durum varieties evaluated, with CDC Teal displaying a higher proportion of fertile, microspore-derived plants compared to CDC Bounty and AC Barrie (Table 4). All plantlets derived from AC Avonlea were sterile, suggesting that spontaneous doubling is not occurring in that variety. This may however not be true for all durum varieties as only a singe variety was evaluated. Given the variable nature of spontaneous doubling in the spring wheat genotypes some mechanism of measuring cellular DNA content could have been used to identify those

plants that are true doubled haploids. Alternatively, fertility is a cheap alternative for determining if plants indeed have doubled chromosomes.

Table 4. Average percentage of fertile plants obtained from microspore culture derived plantlets Fertility was used as a measure of spontaneous doubling of chromosomes. Results are the mean from three separate extractions (replications).

Variety	% Fertility
CDC Teal	51
AC Barrie	21
CDC Bounty	18
AC Avonlea	0
LSD(0.05)=8.1%	

STUDY CONCLUSIONS AND RECOMMENDATIONS:

(A) A useable microspore culture system was developed for spring wheat. The recommended protocol (as outlined in detail in the materials and methods) includes extraction of microspores at the mid-late uni-nucleate stage, following pre-treatment of wheat spikes at 33°C for a minimum of 48 hours. However, as observed in this study, some wheats performed better than others using this protocol, making the protocol less amendable for use in routine breeding (difficult to predict the size of the DH population that would be expected). The protocol however could be used for generating a few targeted populations and/or genetic studies where homozygous lines are an asset for multiple-environment testing.

(B) Durum wheat appears to be more recalcitrant, and further optimization of the microspore culture protocol used in this study is required for successful implementation in plant breeding programs. In this study, optimization studies were conducted only with AC Avonlea, but similar results were obtained for Strongfield in selection experiments (see section 3.2)

(C) Spontaneous doubling of chromosomes was observed in tissue culture experiments for the spring wheats, but not for AC Avonlea. CDC Teal had a higher rate of spontaneous doubling than AC Barrie and CDC Bounty, suggesting that some spring wheat genotypes are more genetically predisposed to spontaneous doubling using the microspore culture protocol used in this study. Given these results, users of the protocol will need to determine the extent of spontaneous doubling on a case by case basis. Some method of DNA quantification could be used to select only those plants that have undergone chromosome doubling to ensure that only those plants with doubled chromosomes are advanced.

3.2 Tissue Culture Selection Experiments

Our first experiments were established to determine if DON could be used as an *in vitro* selection agent to prevent further embryonic development (i.e callus formation) of microspores extracted from FHB resistant and susceptible cultivars of wheat. Initial selection experiments were conducted using logarithmic increases in concentrations of DON in the NPB99 culture medium. At 0 mg L^{-1} of DON, embryonic microspores developing into callus were observed for all wheat genotypes, but a significantly lower percentage was observed in Strongfield compared to spring wheat genotypes (Table 5). These results confirm those from the optimization study that durum wheat varieties do not perform as well as spring wheat in microspore culture. At 0.001 mg L^{-1}, the lowest dose of DON used, the number of embryonic microspores decreased dramatically when compared to the untreated control (Table 5). For all spring wheat genotypes tested, increasing the concentration of DON past 0.1 mg DON L^{-1} prevented microspores from becoming embryonic (Table 5), indicating that DON is a potent inhibitor of embryogenesis in wheat.

Table 5. Percentage of embryonic microspores with visual signs of callus development (±SD) 20 days after treatment with increasing concentrations of DON. Data are the average of two experiments with two replications in each.

[DON] mg L^{-1}	CDC Teal	AC Barrie	ND2710	Strongfield	Snowbird
0	80.8 ± 9.7	81.5 ± 4.9	76.3 ± 10.2	50.8 ± 6.0	78.0 ± 9.1
0.001	47.3 ± 5.9	45.0 ± 4.2	47.3 ± 7.5	24.0 ± 5.5	33.3 ± 10.9
0.01	40.8 ± 8.3	38.5 ± 6.6	41.3 ± 4.5	19.0 ± 9.1	29.8 ± 9.8
0.1	2.0 ± 0.8	1.0 ± 0.8	1.0 ± 0.8	0.0 ± 0.0	1.3 ± 1.0
1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1000	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

However, when expressed as a percentage of embryonic microspores not treated with DON, the FHB resistant genotype ND2710 had a higher percentage of embryonic microspores compared to the susceptible wheat lines CDC Teal, Snowbird, and Strongfield when treated with 0.001 and 0.01 mg L^{-1} of DON (Table 6). These differences were significant ($p<0.05$) when comparing ND2710 to Strongfield and Snowbird, but not when comparing to CDC Teal. In conclusion, the

drastic drop in the number of embryonic microspores developing into calli when DON is included in the culturing medium, coupled with the inability to statistical differentiate FHB resistant and susceptible types clearly suggests that microspores may not be the best materials for practicing selection.

Table 6. Number of embryonic microspores developing into callus (±SD) 21 days after treatment with increasing concentrations of DON expressed as a percentage of embryonic microspores observed in the untreated control (0 mg L^{-1} DON). Data are the average of two experiments with two replications in each.

[DON] mg L^{-1}	CDC Teal	AC Barrie	ND2710	Strongfield	Snowbird
0.001	51.5 ± 4.6	55.3 ± 5.5	59.4 ± 9.3	37.9 ± 9.9	42.4 ± 13.2
0.01	47.6 ± 6.8	47.1 ± 6.2	56.7 ± 10.1	39.1 ± 17.4	37.5 ± 8.5
0.1	2.3 ± 0.8	1.3 ± 1.1	1.4 ± 1.0	0.0 ± 0.0	1.5 ± 1.1

Others have had better success with anther culture and microspore-derived plantlets (Bruins et al. 1993) so it was decided to try optimization using microspore culture derived plantlets.

Three separate experiments were conducted to examine the effects of logarithmic increasing concentrations of DON on survival of plantlets derived from microspore culture. Wheat cultivar "Maringa" was included in this study as it has improved resistance to DON compared to other wheat cultivars (Somers et al. 2003), perhaps making it more amendable for DON selection experiments. No significant differences were detected between experiments (Table 7), suggesting that results were consistent from experiment to experiment.

Table 7. Analysis of variance for plantlet survival in the presence of increasing concentrations of DON (mg L^{-1})

Source	df	Mean Square	F	P
Experiment	2	10.9	7.0	ns
Rep(Experiment)	3	1.6	0.51	ns
[DON]	4	2133.6	1052.74	***
Genotype	4	82.2	15.11	***
Genotype X [DON]	16	26.4	5.95	***
Experiment x Genotype	8	5.5	1.23	ns
Experiment x [DON]	8	2.0	0.46	ns
Experiments x Genotype x [DON]	32	4.4	1.46	ns
Error	72	3.1		

As expected, increasing the concentration of DON had a significantly negative effect on the survival of plantlets (Table 7), and resulted in a severe reduction in the number of surviving plantlets at 1 mg L^{-1} of DON (Table 8), regardless of genotype tested. A significant genotype response to DON treatment was detected (Table 7). When comparing variety means, it was evident that the number of surviving plantlets was variable among varieties, even in the absence of DON as a selective agent (Table 8). In the absence of DON, Strongfield durum wheat had the lowest number of surviving plantlets. This was not unexpected, given the poor performance of AC Avonlea in optimization experiments. In these treatments, plantlets did not develop (revered back to callus), displayed an albino phenotype (lack of chlorophyll production in the leaves), or simply died. Similarly, for ND2710 and Maringa, some plantlets failed to develop into healthy green plants in the absence of DON (Table 8). We are not sure why the plantlets did not survive in these genotypes, but we have noticed that Strongfield, Maringa, and ND2710 are generally recalcitrant in tissue culture. In fact, it was extremely difficult to obtain a sufficient number of microspores-derived plantlets from these lines to conduct these experiments. In contrast, the majority of microspore derived plantlets from AC Barrie and CDC Teal survived in the absence of DON (Table 8). These genotypes generally performed well in microspore culture.

Table 8. Number of surviving haploid plantlets after growing on MS media containing variable concentrations of deoxynivalenol (DON; mg L^{-1}) for 30 days. Each replicate contained 25 germinated plantlets at the onset of the experiment.

	Deoxynivalenol (mg L^{-1})				
Genotype	**0**	**1**	**10**	**100**	**1000**
AC Barrie	23.5	9.3	1.2	0	0
CDC Teal	24.7	11.6	2.5	0.8	0
Maringa	19.0	10.7	5.3	1.1	0.3
ND2710	19.3	7.8	3.2	0.5	0
Strongfield	14.3	3.3	0.5	0	0
DON Mean	**20.2**	**8.6**	**2.5**	**0.5**	**0.1**

$LSD_{0.05}$ for comparison of Treatment means= 2.9.

There was a significant Genotype x DON concentration interaction (Table 7), suggesting that one or more genotypes responded differently to increasing concentration of DON. Strongfield was clearly the most sensitive to DON in the growth media, with decreasing plantlet survival with each incremental increase in DON concentration (Table 8). For CDC Teal, the number of surviving plantlets decreased with each incremental increase in DON concentration,

but not to the same extent as that seen in ND2170 and AC Barrie. Maringa was less sensitive to DON than other genotypes tested (Table 8), with the smallest decrease in surviving plantlets between 1 and 10 mg L-1 DON. CDC Teal, which is susceptible to FHB, also appeared to be les sensitive when compared to AC Barrie and ND2710 (Table 8).

To adjust for variation in plantlet survival in the zero DON control, DON survival scores were standardized relative to the zero DON control to determine the concentration resulting in a 50% reduction in survival (LD_{50}) (Figure 4). Using this analysis, Strongfield plantlets were the most susceptible to DON, with an average LD_{50} of 0.3 mg L^{-1} (Figure 4). ND2710 and AC Barrie had similar DON LD_{50} (0.5 mg L^{-1}), suggesting that they have similar levels of tolerance to this toxin in growth media (Figure 4). CDC Teal had a higher LD_{50} than ND2710 (resistant to FHB) and AC Barrie (intermediate tolerance to FHB) (Figure 4).

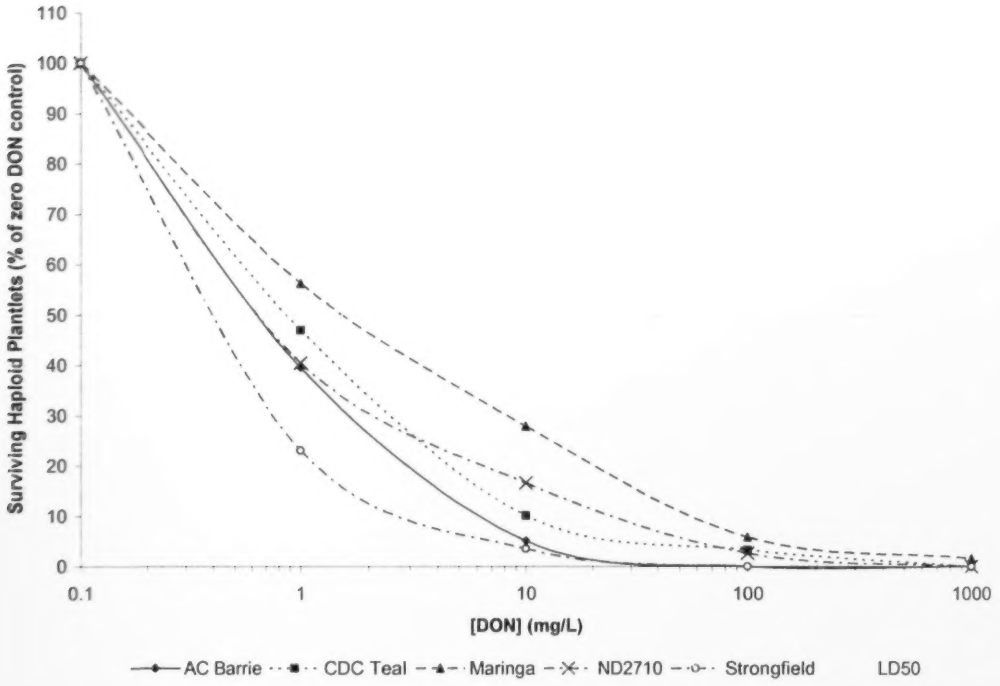

Figure 4. Dose response curve based on plantlet survival after treatment with increasing concentration of deoxynivalenol (DON). The LD_{50} represents the dose where 50% of plantlets were killed.

Taken together, these results suggest that resistance to DON does not correlate with field expressed resistance, given that CDC Teal is susceptible to FHB. However, Maringa had the highest LD_{50} of DON (1.5 mg L^{-1}) (Figure 4). Maringa has been reported to possess resistance to DON (Somers et al., 2004) and our results suggest that Maringa also possesses resistance to DON in tissue culture experiments. Based on these results, a concentration of 1.5 mg L^{-1} of DON in MS media is sufficient to differentiate Maringa from the other wheat genotypes used in this study. However, at that concentration, less than 40% of the Maringa survived DON application. This coupled with the variable nature of wheat varietal perform observed in our experiments suggests using this method for routine selection is limited. As such, a more simple test still needs to be developed, that will limit the confounding effects of tissue culture.

In the last year of this study, we conducted a coleoptile screening assay in the presence of DON to determine if this would be a viable approach for spring and durum wheat screening. Analysis of variance indicated significant varietal differences in coleoptile length (Table 9). Averaged over all DON treatments, Commander had reduced coleoptile lengths compared to other wheat cultivars (Table 10).

Table 9. Analysis of variance for coleoptile length (mm) for 9 wheat and durum varieties in response to increasing concentrations of DON.

Source	DF	Adj MS	F-value
DON Concentration	4	247.658	65.72**
Variety	8	43.656	11.58**
DON Concentration*Variety	32	4.949	1.31ns
Error	135	3.769	

Table 10. Average coleoptile length (mm) in response to increasing concentrations of DON mg L^{-1} for 9 wheat and durum varieties.

[DON]	CDC Teal	Snow-bird	AC Barrie	ND-2710	Maringa	AC Avonlea	Strong-field	Commander	DT735
0	22.5	21.2	23.0	24.8	23.3	24.8	25.8	20.3	24.5
5	21.5	20.7	23.2	22.5	23.5	23.3	23.8	19.8	24.3
10	19.8	19.5	22.8	23.0	21.5	22.0	21.5	18.5	22.8
15	17.9	19.5	19.7	18.3	19.5	18.5	18.6	16.3	18.5
20	18.1	15.9	16.3	19.3	19.5	17.3	17.1	12.3	18.3

$LSD_{0.05}$ for comparing treatment effects = 2.9 mm

These results were expected given that Commander possesses the *Rht-B1* dwarfing gene, which is known to result in reduced coleoptile length. However, the majority of variation in the experiment was attributed to DON concentration treatments (Table 9). Significant decreases in coleoptile length were noted for all varieties at concentrations of 15 and 20 mg DON L^{-1}. If the coleoptile length assay is suitable as a selection tool, the Variety x DON concentration interaction would be significant as that would indicate differential varietal response to increasing rates of DON (i.e. resistant types would not be affected by DON whereas susceptible types would). However, that interaction was found not to be significant (Table 9). For example, ND2710 (resistant) and AC Barrie (intermediate resistance) had, on average, a 5-7 mm reduction in coleoptile length, compared to CDC Teal (susceptible) which also had a 5 mm reduction at 20 mg DON L^{-1} (Table 10). There were sufficient degrees of freedom (df=135) to detect biologically significant interactions (Table 9). The fact that no interaction was detected suggests that this assay will not be effective as a selection tool for FHB resistance in wheat.

STUDY CONCLUSIONS AND RECOMMENDATIONS:

(A) Selection with DON at the microspore level (post embryogenesis) resulted in a dramatic decrease in the number of embryonic microspores and calli. Lines with moderate-high levels of FHB tolerance (ND2710 or AC Barrie) could not be distinguished based on survival of embryonic microspores.

(B) Inclusion of DON in plant regeneration media resulted in a significant reduction in green plantlets, regardless of genotype evaluated. A higher percentage of Maringa plants survived DON treatment, but at concentrations that reduced surviving plantlets below a level where it would be economically feasible to use the protocol.

(D) Screening for coleoptile resistance with DON *in vitro* also could not adequately differentiate FHB resistant and susceptible types. As such, this protocol is not recommended for routine screening in wheat breeding programs. The results from our selection experiments do seem to suggest that factors other than DON are contributing to *F. graminearum* virulence. This is supported by recent *F. graminearum*-wheat host pathogen interaction studies that shown that the interaction is far more complex than just DON alone (Goswami et al. 2006).

(E) Our results suggest that an *in vitro* screening protocol has limited application in breeding FHB resistance for wheat. Molecular marker assisted selection coupled with field screening will

still remain to be the best approach for breeding resistance. However the microspore culture protocol developed for spring wheats as part of this proposal could be used by breeders to develop haploid plantlets from segregating populations for DNA analysis. This approach seems most practical as only plants with putative resistance genes would be generated into doubled haploid plants. Furthermore, knowledge of the resistance genes segregating in the population would allow breeders to predict the number of plantlets that would be required to achieve an adequate population size for selection of other important traits.

3.3 Evaluation and Characterization of Novel Sources of FHB Resistance for Durum Wheat:

The ADF grant approved to conduct this research only covered 50% of the cost, with the remaining 50% being covered by Industry funds. As part of this project and to fulfill our industry commitment, a research effort was initiated to identify and characterize novel sources of FHB resistance for durum wheat. Based on preliminary field trials conducted in 2001, we identified four accessions of *T. turgidum* ssp. *dicoccoides* with putative FHB resistance, namely TG13205, TG13475, TG3487 and TG42074. As part of this project, we re-evaluated these accessions in field and greenhouse trials and examined segregating populations derived from crossing the TG accessions with adapted durum wheat cultivars.

In replicated experiments, three of these accessions TG13205, TG13475, and TG3487 consistently displayed Type II resistance similar to ND2710 in both field and greenhouse trials (Table 11). The fourth accession, TG42074, appeared to have only moderate Type II resistance, similar to AC Barrie (Table 11). Based on these results, all four accessions have a marked improvement in FHB resistance compared to durum wheat cultivar Strongfield and represent an excellent source of genetic resistance to this disease. To further investigate resistance, crosses between Strongfield and each of the four resistant accessions were generated and F_1 plants from each cross were evaluated for FHB resistance with appropriate controls (Table 12). The FHB scores of F_1 plants from all four crosses were intermediate between the susceptible and resistant parents, suggesting resistance is governed by additive gene action in all four accessions (Table 12).

Table 11. Mean FHB score (0-5) for four *T. dicoccoides* accessions and resistant (ND2710) intermediate resistance (AC Barrie), and susceptible (CDC Teal, DT712) checks. A score of zero represents no fungal spread, whereas a score of 5 represents >90% of the spike infected. Data is the average of eight FHB scores.

Accession	Field[a]	Greenhouse[a]
TG13205	2.1 a	1.1 a
TG13475	1.7 a	0.8 a
TG3487	1.8 a	1.0 a
TG42074	1.7 a	2.9 b
CDC Teal	4.3 b	4.6 d
Strongfield	4.7 b	4.8 d
AC Barrie	3.8 ab	3.4 b
ND2710	2.4 a	1.5 a

[a]Means followed by the same letter are not significantly different at the 5% significance level

Table 12. Mean FHB score (0-5) of four accessions with FHB resistance, Strongfield, and F_1 populations. A score of zero represents no fungal spread, whereas a score of 5 represents >90% of the spike infected. Data presented represents the mean(±SD) based on observation of five artificially inoculated plants.

Genotype	FHB Score	F_1 Population	FHB SCORE
Strongfield	4.8±0.3		
TG13205	1.4±0.8	TG13205/Strongfield	2.7±0.6
TG13475	1.2±0.9	TG13475/Strongfield	2.9±1.0
TG3487	1.9±1.0	TG3487/Strongfield	2.7±0.9
TG42074	2.2±0.9	TG42074/Strongfield	3.8±0.8

Additive gene action is the form of gene action most desirable to plant breeders, as heterozygous genotypes can be more easily distinguished from homozygous resistant breeding lines, resulting in improved selection response in segregating generations. The lower F1 scores relative to Strongfield also confirm that resistance is in fact heritable.

DT735, a breeding line developed by AAFC-SPARC, has consistently shown improved FHB resistance in our field trials (Figures 5 and 6) over other durum wheat cultivars, like AC Morse. As such, each of the TG accessions were crossed with DT735 and generated $F_{2:3}$ families for field evaluation. In 2005 field testing, the four TG accessions displayed FHB indices above the susceptible cultivars CDC Teal and AC Morse (Figures 5 and 6), confirming our previous greenhouse and field testing (Table 11) that these accessions have improved FHB

resistance. TG3487 possessed the lowest FHB index relative to the other TG accessions and AC Morse (Figure 5 and 6), consistent with greenhouse testing (Table 11).

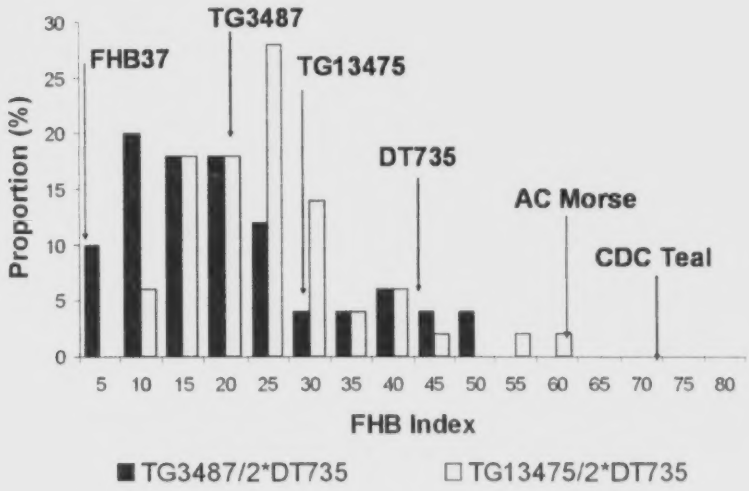

Figure 5. Frequency distribution of FHB indices for $F_{2:3}$ families from backcross populations derived from crosses TG3487/2*DT735 and TG13475/2*DT735. AC Morse and CDC Teal are susceptible to FHB and FHB37 was used as a resistant control. DT735 is a breeding line with intermediate tolerance to FHB.

Evaluation of the $F_{2:3}$ families indicated transgressive segregation for FHB resistance in all four populations (Figures 5 and 6). Transgressive segregation describes a situation where progeny from a cross have resistance greater/less than that observed in the resistant and susceptible parents, respectively. Transgressive segregation for FHB resistance has been reported in a number of other research studies as well (Somers et al., 2003; Ma et al. 2006). Evidence of transgressive segregation suggests that all four of the TG accessions possess novel FHB resistance genes to those in DT735. This is the desirable situation as it implies that pyramiding resistance genes from any one of the TG accessions with the resistance from DT735, will result in resistance higher than that observed in DT735 and currently registered durum wheat varieties.

The negative relationship between plant height, days to heading and FHB tolerance is well known (for example see Somers et al. 2004). Correlations between FHB indices and plant height were negative in all four populations, but no correlations were observed with FHB indices and days to heading (Table 13).

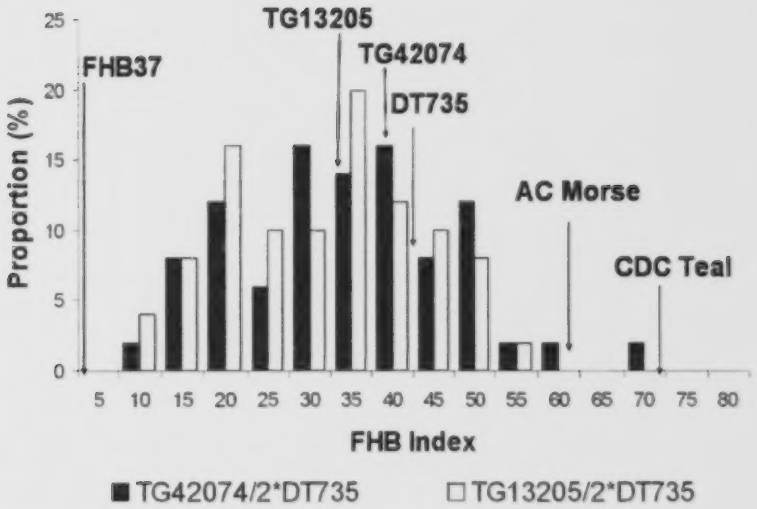

Figure 6. Frequency distribution of FHB indices for $F_{2:3}$ families from backcross populations derived from crosses TG42074/2*DT735 and TG13205/2*DT735. AC Morse and CDC Teal are susceptible to FHB and FHB37 was used as a resistant control. DT735 is a breeding line with intermediate tolerance to FHB.

These results suggest that plant height may have a pleiotropic effect on the expression of resistance in this population. This is a negative relationship as it implies that by selecting shorter types (desirable), FHB indices of selections would increase. However, we were able to identify families with plant height similar to AC Morse and FHB indices below 20. We selected 10 individual plants with acceptable plant height and good FHB resistance from each of 10 of the best $F_{2:3}$ families from each cross. These lines were evaluated in the rust nursery in 2006 and lines selected for good leaf/stem rust resistance selected for evaluation in F_5 head rows in 2007.

Table 13. Correlation coefficients between FHB Indices and plant height and days to heading for $F_{2:3}$ families form four backcross populations.

Cross	Days to Heading[a]	Plant Height[b]
TG3487/2*DT735	-0.13ns	-0.36**
TG13475/2*DT735	-0.21ns	-0.34**
TG42074/2*DT735	-0.19ns	-0.32**
TG13205/2*DT735	-0.18ns	-0.29**

[a] ns=not significant at the 5% significance level [b] ** = correlation coefficient was highly significant with $p<0.001$.

Differences in the frequency of $F_{2:3}$ families with low FHB indices (more resistant) were noted among the four populations, but in all cases, $F_{2:3}$ families were observed with FHB indices well below the most resistant parent of the cross population (Figure 5 and 6). The $F_{2:3}$ families from the cross TG3487/2*DT735 produced the highest proportion of families with FHB indices approaching levels similar to that seen in FHB37 (Figure 5), a hexaploid wheat with excellent Type II FHB resistance. As such we further evaluated a population of approx 400 F_5 lines from the cross TG3487/2*DT735 in an FHB screening nursery in 2006. A second population, consisting of approx 400 lines derived from crossing of Strongfield and DT735 was also evaluated for comparison. Repeated check cultivars (Strongfield, AC Morse, CDC Teal, FHB37 and DT735) were included through out the field nursery to evaluate spatial variation in scoring for FHB infection/severity. TG3487 data was lost because of uneven germination. In 2006 field testing, the susceptible checks AC Morse and CDC Teal had significantly higher FHB scores than FHB37 whereas DT735 was intermediate in resistance (Figure 7). As seen with the $F_{2:3}$ family data, considerable variation for FHB resistance was evident in the F_5 lines from the cross TG3487/2*DT735. Approx. 18% of the lines had an FHB index $\leq$15, well below the indices observed in AC Morse and CDC Teal (Figure 7). The Strongfield/DT735 F_5 population was less variable, but it was interesting to note transgressive segregation in that cross. This implies that Strongfield is also contributing FHB resistance to the F5 progeny. In 2007, F6 lines from the same population will be examined for resistance to confirm this data, and if it holds, we will focus efforts to genetically map this population and perform QTL analysis for FHB resistance. Given that both Strongfield and DT735 are adapted to western Canada, selections out of this cross have been advanced a generation in New Zealand for repeated testing in 2007 and some lines will be used for further crossing. Furthermore, we also have top crossed Strongfield on to the TG3487/2*DT735 cross (Table 14) in hopes of combined the apparent FHB resistance from Strongfield, with that from DT735 and TG3487.

Table 14. Crosses developed to incorporate resistance from TG3487 into adapted durum wheat cultivars.

Cross Code	Pedigree	Stage in Breeding Program
D05.35	DT749/TG3487/2*DT735	F4 lines generated
D05.36	DT712//TG3487/2*DT735	F4 lines generated
D06.70	DT712/[TG3487/2*DT735 F3 sel]	F1 population Inc in NZ*
D06.71	DT760/[TG3487/2*DT735 F3 sel]	F1 population Inc in NZ*

*NZ=New Zealand

As with the 2005 data, no significant ($p>0.05$) correlations were observed between FHB indices and days to heading, but a significant association was observed for FHB index and plant height in the TG3487/2*DT735 population ($r=-0.41$; $P<0.05$). These results confirm the data from 2005 that illustrated that variation for height in that population is contributing to variation in FHB reaction in this population. Given the continued expression of type II FHB resistance observed in TG3487, we have developed a number of crosses to transfer resistance to adapted cultivars (Table 14). We have also made an F_4 selection out of the cross TG3487/2*DT735 that has acceptable plant height, durum spike morphology and improved resistance over TG3487 for further crossing to Strongfield (DT712) and DT760, an extra-strong gluten line developed by Dr. J. Clarke, AAFC. The F1 plants from these crosses are being increased in New Zealand this winter and F_2 single plant selections will be made in the summer of 2007. We have recently received additional ADF funding to map resistance in recombinant inbred lines from the cross TG3487/2*DT735, and these crosses (Table 14) could be used for QTL validation.

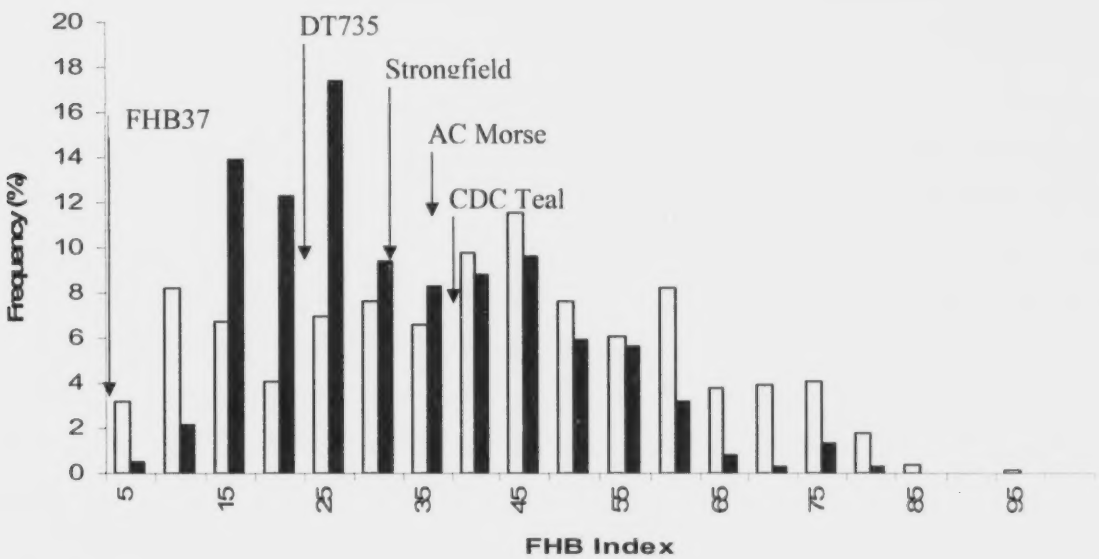

Figure 7. Frequency distribution of FHB indices for F5 lines from two populations segregating for FHB resistance derived from crosses TG3487/2*DT735 (grey bars) and DT712/DT735 (black bars). AC Morse and CDC Teal are susceptible to FHB and FHB37 was used as a resistant control. DT735 is a breeding line with intermediate tolerance to FHB. Strongfield has shown consistently better resistance to FHB than AC Morse in PRRCG registration trials. Values of the check cultivars are the average of 20 observations. The average standard deviation

of susceptible check cultivars was 9.8, whereas the standard deviation for FHB37, a resistant type was 2.4.

As expected for wild *T. dicoccoides* accessions, the seed morphology of the TG lines is quite unique from that of commercially acceptable durum wheat varieties. In fact, all four of the TG lines are red-seeded types, leading to the hypothesis that compounds could be present in the seed coat which maybe in part responsible for the elevated tolerance of these accessions. Plant phenolic compounds have long been associated with the role of disease resistance in plants (Nichelson et al., 1992) and in wheat bran, ferulic acid constitutes up to 90% of the total phenolic acids (Abdel-Ali et al. 2001). Interestingly, McKeehen et al. (1999) demonstrated that ferulic acid inhibits the growth of Fusarium species *in vitro*, and noted that wheat lines with elevated FHB resistance had higher FAC. Similar results have been observed in maize (Bakan et al., 2003; Bily et al., 2003). Although not part of the original proposal, an experiment was conducted to determine if there were differences in ferulic acid concentration (FAC) among the TG lines in relation to DT735 and Strongfield.

For this experiment, grain samples were analyzed for FAC at 15 days post anthesis (dpa), a time when FA is at its maximum (Abdel-Aal et al., 2001) and the time at which cereals are most prone to FHB infection. Samples were collected and analyzed again once plants had reached physiological maturity. Regardless of accession evaluated, FAC were higher at 15 dpa than in the mature grain (Table 15). Relative to Strongfield and AC Avonlea, all of the "TG" accessions had significantly higher FAC at both sampling times (Table 15).

Table 15. Average ferulic acid content (FAC; mg g^{-1}) and relative concentration of an unknown compound at elution time 16.6.

Cultivar	15 dpa		Physiological maturity	
	FAC	Unknown	FAC	Unknown
TG3487	985	313	735	380
TG42074	829	201	544	334
TG13475	887	220	698	310
TG13205	828	134	641	160
Strongfield	801	131	514	181
AC Avonlea	818	138	522	191
DT735	1072	292	599	308
LSD(0.05)	17	9	18	7

TG3487, the most resistant of the TG accessions (Table 15), had the highest FAC of the TG accessions, while TG42074, the TG accession with the least field resistance (Figure 6) had the lowest (Table 15). Strongfield and AC Avonlea had the lowest FAC at 15 dpa. Interestingly, DT735, which has displayed better FHB tolerance (Table 11), also had a significantly higher FA content at 15 dpa when compared to Strongfield and AC Avonlea (Table 15). Similar trends were observed at physiological maturity with DT735 and TG3487 having significantly higher FAC than Strongfield and AC Avonlea. In all lines, an unknown peak was detected at time 16.4, near the ferulic acid peak at 16.6 (Figure 8).

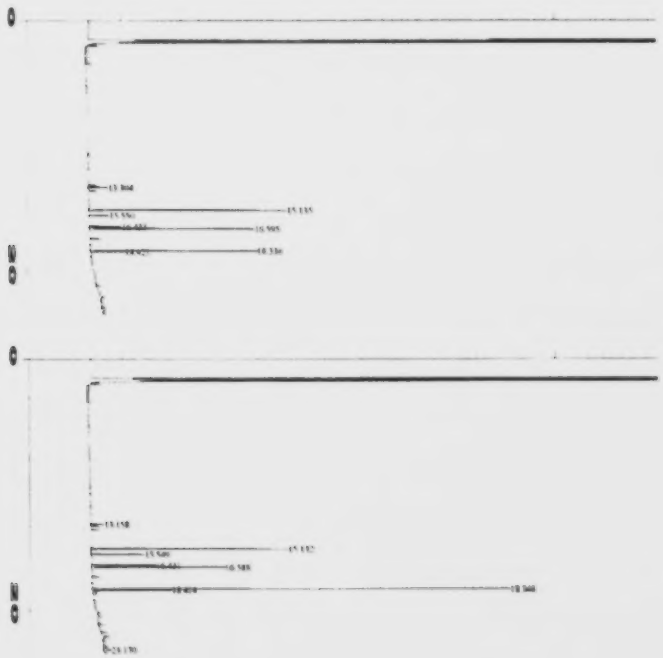

Figure 8. Example of phenolic acid profiles generated using HPLC from samples collected 15 dpa. Numeric values represent time to elution (minutes). An unknown compound was detected at 16.4, near the ferulic peak at 16.6 in all samples analyzed. For comparison, the top chromatogram is Strongfield and the bottom is TG3487.

We are not certain what this compound is, but it could be a metabolite of ferulic acid found naturally in wheat. It is more likely however that this is ferulic acid-derived artefact that was induced during the extraction and separation process. Similar to ferulic acid, the unknown compound was highest in TG3487 and DT735 (Table 15). Thus our results appear to agree with

those of McKeehen et al. (1999) that FAC is elevated in lines with higher FHB resistance. However, since only seven lines were evaluated the apparent association of FAC with FHB resistance should be interpreted with caution and further testing is required to confirm if this association is real. We will be performing genetic mapping and QTL analysis for FHB in a set of recombinant inbred lines derived from the TG3487/2*DT735 population and hope to map FAC as well. This will ultimately confirm if elevated FAC is in fact associated with FHB resistance. If there is an association with resistance, screening for FAC could be a viable alternative for FHB screening in our durum wheat breeding program.

As indicated in the introduction, one strategy to improve the FHB resistance in durum wheat is to introgress known resistance genes from spring wheat. This is possible as durum wheat shares two common genomes (A and B) with spring wheat. We used a marker assisted selection breeding to transfer the *Fhb1* (Cuthbert et al. 2006) from ND2710 into durum wheat using the cross DT513/ND2710//Strongfield. Approx. 90 F_5 lines derived from this cross were grown in hill plots at Saskatoon and selected for rust resistance and durum wheat phenology. Approx. 30 lines were identified and subjected to molecular marker analysis with wheat microsatellite markers *gwm533* and *gwm49,* two markers that flank the *Fhb1* resistance locus. Selection with both flanking markers would ensure successful transfer of the *Fhb1* locus. A total of six lines produced the DNA banding pattern expected (similar to ND2710) for the presence of *Fhb1* (Figure 9).

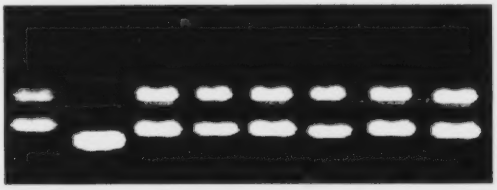

Figure 9. Selection of durum wheat lines containing *Fhb1* with *gwm533* (bottom amplification in Lane 1) and *gwm493* (top amplicon in Lane 1). Lane 1 is ND2710 (which possesses *Fhb1*) and Lane 2 is Strongfield. The remaining lanes represent durum wheat lines that have the same DNA banding pattern as ND2710 and thus would contain the *Fhb1* locus.

Field evaluation found that these lines had significant lower FHB indices than all of the durum wheat check cultivars evaluated. A recent presentation at the 2006 Crop Science Society of America annual meetings by S. Bhamidimarri out of North Dakota State University (see abstract online at http://crops.confex.com/crops/20(5am/techprogram/P23456.HTM) also showed that

Fhb1 can be transferred and expressed in a durum wheat genetic background. However, all six lines were a found to be significantly lower yielding than all check cultivars (Table 16), likely because they were extremely early maturing (Table 16). The lines were also noted to have poor grain protein concentration (Table 16). We have since conducted an additional backcross to Strongfield to develop lines for further evaluation, but we have yet to develop sufficient seed for screening purposes.

Table 16. Yield (kg ha^{-1}), days to maturity, grain protein concentration (%)and FHB indices for six durum wheat lines (designated as DC68) and four check cultivars.

ID	Yield (kg ha^{-1})	Maturity (days)	Protein (%)	FHB Index
AC Avonlea	4260	129	14.5	71
Strongfield	4794	129	14.8	45
AC Morse	4144	128	13.8	68
AC Navigator	4124	130	13.0	67
DC68-5059	4227	122	13.5	28
DC68-5066	3972	122	13.2	28
DC68-5068	4068	122	13.8	22
DC68-5086	3904	122	13.2	23
DC68-5088	4278	122	12.1	18
DC68-5093	4207	122	12.6	32
LSD(0.05)	410	2	0.6	21

Study Conclusions and Recommendations:

(A) Four *T. dicoccoides* accessions were identified that in both field and greenhouse trials have improved type II resistance to FHB over existing durum wheat varieties. The best line TG3487 displaying type II FHB resistance equivalent to ND2710 and is recommended as a source of resistance for durum wheat breeders.

(B) Evidence of transgressive segregation involving crosses between the accessions and DT735 suggest that the resistance gene(s) in DT735 are different from those in the TG accessions. Our data also suggests that higher levels of tolerance to FHB can be achieved by stacking the resistance from the TG accessions and DT735. Stacking resistance genes is most desirable as it not only improves FHB resistance levels, but it could also slow breakdown of effective resistance over time. However, breeding becomes increasingly challenging with each additional gene under selection, particularly when each of those genes produces a similar phenotype. As such, we recommend genetic mapping of the resistance in the TG accessions as well as DT735 to

identify molecular markers that would allow plant breeders to more easily select progeny that possess the resistance from both sources.

(C) In durum registration trials it is becoming increasingly evident that Strongfield has better FHB resistance than the other check varieties. In this study we also noted transgressive segregation for FHB tolerance in F_5 lines from the cross Strongfield/DT735. These results suggest that Strongfield has positive genes for FHB resistance, which alone have only a small effect. When combined with the resistance genes from DT735, a high level of FHB tolerance can be achieved.

(D) Ferulic acid concentration (FAC) was higher in the TG accessions and DT735 when compared to Strongfield and AC Avonlea, suggesting and association between these two characters. Although an association between FAC and FHB tolerance was observed, more detailed genetic mapping studies are required to determine if in fact FAC is truly the cause of elevated FHB tolerance in these lines.

(E) Transfer of *Fhb1* from ND2710 to durum wheat using marker assisted selection did result in improved FHB tolerance, but further backcrossing is required to achieve acceptable agronomic performance.

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Personnel Involved in the project:

Paid from this project:

Arryn Hahn: Tissue culture experimentation

Akiko Tomita: Tissue culture, marker assisted selection

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